

Distinguishing black carbon from biogenic humic substances in soil clay fractions

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Received 28 March 2007; received in revised form 6 October 2007; accepted 28 October 2007

Available online 4 December 2007

Abstract

Most models of soil humic substances include a substantial component of aromatic C either as the backbone of humic heteropolymers or as a significant component of supramolecular aggregates of degraded biopolymers. We physically separated coarse (0.2–2.0 μm e.s.d.), medium (0.02–0.2 μm e.s.d.), and fine (>0.02 μm e.s.d.) clay subfractions from three Midwestern soils and characterized the organic material associated with these subfractions using ^{13}C -CPMAS-NMR, DTG, SEM-EDX, incubations, and radiocarbon age. Most of the C in the coarse clay subfraction was present as discrete particles (0.2–5 μm as seen in SEM images) of black carbon (BC) and consisted of approximately 60% aromatic C, with the remainder being a mixture of aliphatic, anomeric and carboxylic C. We hypothesize that BC particles were originally charcoal formed during prairie fires. As the BC particles aged in soil their surfaces were oxidized to form carboxylic groups and anomeric and aliphatic C accumulated in the BC particles either by adsorption of dissolved biogenic compounds from the soil solution or by direct deposition of biogenic materials from microbes living within the BC particles. The biogenic soil organic matter was physically separated with the medium and fine clay subfractions and was dominated by aliphatic, anomeric, and carboxylic C. The results indicate that the biogenic humic materials in our soils have little aromatic C, which is inconsistent with the traditional heteropolymer model of humic substances.

Published by Elsevier B.V.

Keywords: Humic substances; Soil organic matter; Charcoal; Black carbon; humification; Thermogravimetric analysis; NMR spectroscopy; Clay mineralogy; Clay-humic complexes

1. Introduction

Soil organic matter (SOM) greatly enhances soil quality and productivity (Stevenson, 1994). Soil organic matter is a major reservoir of essential plant nutrients (especially N, P, and S), contributes substantially to a soil's cation exchange capacity, and increases the stability of soil structure, thereby facilitating the movement and retention of bioavailable air and water in

soils. Soil organic matter also enhances the soil's ability to act as a filter to retain both organic and inorganic pollutants. And, SOM is a large dynamic pool of organic C that plays a critical role in the global C cycle (Lal, 2004).

Processes responsible for the formation and stabilization of SOM are only partially understood. Fresh biological tissues from plant and animal residues are partially decomposed in soil environments through microbial and extracellular enzyme activity. Most residue biomass is rapidly mineralized and thereby recycled to the atmosphere (primarily as CO_2) or released to the soil solution as bioavailable nutrients (such as NH_4^+ , NO_3^- , PO_4^{3-} , and SO_4^{2-}). However, a small fraction of residue biomass is transformed into a stabilized material that has traditionally been called humus while in soil and humic substances (HS) after extraction from the soil.

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Humic substances have no single composition or structure, however several models depicting average or idealized HS structures have been proposed (Orlov, 1985; Steelink, 1985; Schulten and Schnitzer, 1993; Stevenson, 1994). These traditional HS structures typically described high molecular weight (>10,000 Da) heteropolymers with a backbone of dominantly single but occasionally polynuclear aromatic rings joined by various ether, ester, and aliphatic linkages with numerous terminal hydroxyl and carboxyl groups. Short peptide, oligosaccharide, and fatty acid groups are integrated into the structure through ester, ether, and amide linkages. A few N and/or O hetero-rings may also be depicted. Recently, the traditional understanding of HS has been challenged (Burdon, 2001; Piccolo, 2001; Simpson, 2002), as several authors now describe HS as aggregates of relatively small biopolymers (200–3000 Da) in various stages of degradation. In this aggregate model, hydrogen bonding and weak dispersive forces hold the molecular components of HS together in supramolecular structures. Both the traditional heteropolymer and the recent aggregate models assume that aromatic structures, derived primarily from the degradation of lignin and/or fungal biomass, are a substantial and integral component of soil HS.

A major challenge in determining the nature of HS arises from the difficulty of separating HS from the inorganic soil matrix. Due to their inherent polyfunctionality, HS form strong complexes with soil clay minerals. To overcome this problem, traditional techniques, including the popular International Humic Substances Society (IHSS) method (Swift, 1996), use a strong base extraction followed by acidification of the extract to separate humic acid (precipitate) and fulvic acid (soluble) fractions. Typically, fulvic acids are lower in molecular mass and have more oxygen-containing functional groups, but less N and C per unit mass than humic acid (Stevenson, 1994; and references therein). In spite of the convenience of characterizing humic and fulvic acids, the genetic significance of these procedurally based chemical fractions is questionable.

Both the heteropolymer and the aggregate models of HS structure are further complicated by the recent identification of significant amounts of pyrogenic black C (BC) in soils (Glaser et al., 2002; Skjemstad et al., 2002; Brodowski et al., 2005). Black C originates from the pyrolysis of organic material during fire events or, to a lesser extent, by the deposition of industrial soot (Almendros et al., 2003; Fernandes et al., 2003; Simpson and Hatcher, 2004). Soil BC typically consists of highly carboxylated, linearly fused, and/or highly condensed aromatic polymers (Kramer et al., 2004). Black C is highly stable in soil, with mean residence times derived from radiocarbon dating commonly in excess of 1000 years (Glaser et al., 2002; and references therein). The ability of BC to resist both chemical weathering and microbial attack suggests that BC will accumulate over time in soils where vegetation is subject to periodic fire events. The identification of large amounts of BC in soil leads to a fundamental question: If BC contributes substantially to the total aromatic C content of soil organic matter, then is the aromatic prevalence overstated in the various proposed models of HS structure?

Soil organic matter has long been studied by fractionating soil and characterizing the compounds associated with the various

size fractions. Many investigators have reported that organic matter in clay fractions is compositionally distinct from that in sand and silt fractions (Christensen, 2001). For example, Zhang et al. (1988) investigated organic matter in pairs of cultivated and uncultivated prairie- and forest-derived soils in Iowa. They found that the C:N ratio of soil organic matter decreased as microaggregate sizes decreased from coarse silt to fine clay. Zhang et al. (1988) reported that the ratio of humic acid to fulvic acid was 2–3 times greater in SOM extracted from silt fractions than that extracted from clay fractions. Mao et al. (in press) have recently reported that humic acids associated silt and clay fractions can differ in their structural composition. Clay-fraction HA was shown to contain more readily oxidizable components and aliphatic moieties than did silt-fraction HA. We have used aggressive physical fractionation procedures to separate compositionally unique soil clay subfractions (Laird et al., 2001). However, no methods have reported the physical separation of BC from soil clays.

The objectives of this paper are to describe a method for physically separating BC from soil clays and to characterize the BC and biogenic humic substances in our soils.

2. Materials and methods

2.1. Samples

Three surface (0–15 cm) soil samples were collected from two agricultural fields and a native prairie in Pocahontas County, Iowa. All three soils are classified as Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquoll). The three sampling sites are located within 30 m of each other and within the same soil mapping unit on a nearly level surface, but have been subject to different management histories. Currently both agricultural fields are under a two-year corn/soybean (*Zea mays*/*Glycine max*) rotation with conventional tillage. The prairie site is within the Kalsow Prairie State Preserve and has never been cultivated. A sample of reference charcoal was prepared by heating Canada Wildrye grass (*Elymus Canadensis*) tissue in a muffle furnace at 500 °C for 6 h in an Ar atmosphere.

2.2. Fractionation

Samples of the whole clay (<2 µm e.s.d.) fraction were separated from the soils by sedimentation without chemical pretreatments. To do so, fresh soil was dispersed in deionized water (18 MΩ) and the upper 10 cm of suspension was removed by siphoning after the appropriate settling time. Water removed during the siphoning was replaced and the dispersion-sedimentation-siphoning process was repeated up to 10 times. The whole clay samples were de-watered using an auto-irrigation flask, air-dried, and then crushed in an agate mortar. By exhaustively repeating the dispersion-settling-siphoning process we were able to recover the vast majority but certainly not all of <2 µm fraction from our soils. In this paper we assume that any clay-humic complexes that were not recovered have similar properties to those that were recovered with the whole clay samples.

A portion of the whole clay was saturated with Na^+ by washing three times with 1 M NaCl, dispersed in deionized water by sonication, subjected to high-speed centrifugation (38,724 g for 20 min), and then decanted to separate the 0.02–2.0 μm and <0.02 μm (fine-clay) subfractions. Cycles of dilution-dispersion-centrifugation-decantation were repeated exhaustively until negligible fine-clay was extracted. In the final stage, the 0.02–2.0 μm subfraction was subjected to a series of dilution-dispersion-slow speed centrifugation (387 g for 20 min)-decantation cycles to separate the medium (0.02–0.2 μm e.s.d.) and coarse (0.2–2.0 μm e.s.d.) clay subfractions. During a typical fractionation procedure, a sample was subjected to about 15 dilution-dispersion-centrifugation-decantation cycles. Samples of the whole clay and various subfractions were flocculated by adding CaCl_2 , washed three times with 1 M CaCl_2 , dialyzed against deionized water until excess Cl^- was removed, and then freeze dried. These samples were used for chemical and spectroscopic analysis.

A portion (0.5 g) of the coarse clay subfraction was Na-saturated by washing in 1 M NaCl, suspended in 1.5 g cm^{-3} Napolytungstate (SPT), dispersed by sonication, and centrifuged at 1075 g for 100 min. Material floating on the SPT solution or adhering to the upper walls of the centrifuge tube was removed and is hereafter referred to as the $<1.5 \text{ g cm}^{-3}$ density fraction of the coarse clay subfraction.

Quantitative particle size analysis (Gee and Bauder, 1986) was performed by a combination of wet sieving to separate the sand ($>53 \mu\text{m}$) and the pipette method to determine the proportions of silt (2–53 μm e.s.d.) and whole clay ($<2 \mu\text{m}$ e.s.d.) in the soils. Quantities of coarse, medium, and fine clay in the soils were estimated by multiplying the percent clay determined by the pipette method by the relative quantities of the coarse, medium, and fine clay subfractions recovered during the aggressive Na-saturation-sonication-centrifugation procedure described above.

2.3. Analyses

Total C and N were determined by high temperature combustion using a Carlo Erba NA1500 NSC elemental analyzer (Haake Buchler Instruments, Paterson, NJ).

Samples of the Ca-saturated whole clay and coarse, medium, and fine clay subfractions were analyzed by ^{13}C -cross polarization magic angle spinning-nuclear magnetic resonance spectroscopy (^{13}C -CPMAS-NMR) at 50 MHz (C frequency). Spectra were obtained on a Chemagnetics CMX-200 MHz proton frequency spectrometer equipped with a 7.5-mm Chemagnetics ceramic probe. The samples were packed in zirconia rotors and spun at 5000 Hz. The acquisition parameters were 1 ms contact time, 1 s pulse delay, and 4.5 ms (90 degree) pulse (Wershaw et al., 2005).

Samples of the coarse and fine clay subfractions were analyzed for radiocarbon age using an accelerator mass spectrometer with $\delta^{13}\text{C}$ correction to -25 . Radiocarbon dating was performed by Bata Analytic, Inc. Miami FL.

The relative bioavailability of C in the Ca-saturated whole clay and various clay subfractions was assessed using 28-day aerobic incubations at $25 \pm 1^\circ\text{C}$. Samples (5.00 g) were mixed with 20 g of acid-washed quartz sand and 5.00 mL of a 0.01 M

KH_2PO_4 (pH=6.5) buffer extract of a freshly collected field moist soil sample. During the incubation, evolved CO_2 was trapped in alkali and quantified by titration using a phenolphthalein indicator. The procedure is similar to that described by Drinkwater et al. (1996). Three replications were independently prepared, incubated, and analyzed for each sample.

The mineralogy of the samples was determined by X-ray diffraction (XRD) using a Siemens D5000 diffractometer operated in the Θ – Θ mode equipped with a Li(Si) detector. Portions (100 mg) of the Ca-saturated samples were slurried in 95% (v/v) ethanol, oriented on glass slides by the paste method, air dried, and analyzed between 2 and $32^\circ 2\Theta$ using Cu $\text{K}\alpha$ radiation.

Samples of the coarse and fine clay subfractions for one of the agricultural soils (field 1) and reference samples were analyzed by differential thermal gravimetry (DTG) using a Seiko Model 320 TG/DTA. Samples of the Ca-saturated, freeze-dried, clay subfractions (20 mg) were run in air with a heating rate of 10°C per min. Reference organic materials (lipid, cellulose, lignin, and charcoal) were physically mixed (1:10 w:w) with silt-size quartz before thermal analysis of 20 mg of these prepared samples as above.

Samples were analyzed by scanning electron microscopy (SEM) using a JOEL (Japan Electron Optics Laboratory, Peabody MA) JSM 5800 LV at 10 KV. The Ca-saturated clay subfractions and the $<1.5 \text{ g cm}^{-3}$ density fraction of the coarse clay subfraction were prepared for SEM analysis by dispersing the samples in deionized water and transferring one drop of suspension to silicon wafers mounted on SEM studs. Some samples were sputter-coated with Au/Pd to reduce surface charging and enhance image quality. Uncoated samples were analyzed by both secondary electron imaging and energy dispersive X-ray (EDX) analysis using a Kevex Instruments Quantum EDX detector (Thermo Fisher Scientific, Inc., Waltham, MA). The necessity of using uncoated samples and removing the final SEM aperture to obtain EDX spectra degraded image quality.

3. Results and discussion

Soil organic matter (SOM) in the sand and silt fractions of the soils is a mixture of biomass, particulate organic matter (Cambardella and Elliott, 1992; Christensen 2001), and humus associated with stable mesoaggregates ($>20 \mu\text{m}$) that remain intact during the dispersion-sedimentation process. Organic C associated with the sand and silt fractions represents 40 to 53% of total soil organic C (SOC) for our samples (Table 1). Our

Table 1
Distribution of mass, C and N in the physical fractions of the studied soils

Fraction	Mass (g kg^{-1} soil)			Organic C (g kg^{-1} soil)			Organic N (g kg^{-1} soil)		
	Field 1	Field 2	Prairie	Field 1	Field 2	Prairie	Field 1	Field 2	Prairie
Sand	365	450	390	2.5	3.7	4.1	0.19	0.15	0.21
Silt	318	247	347	13.8	8.9	17.4	1.33	0.54	1.29
Coarse clay	90	69	110	5.8	4.7	7.2	0.25	0.19	0.34
Medium clay	111	102	97	8.3	8.0	7.8	0.63	0.50	0.66
Fine clay	116	132	56	6.6	6.0	4.0	0.51	0.32	0.29
Whole soil	1000	1000	1000	37.1	31.3	40.5	2.91	1.71	2.79

Table 2

Chemistry, radiocarbon age (C minimum mean residence time), and percent (w:w) of total C mineralized during 28 day aerobic incubations for organic matter associated with the whole clay fraction and the coarse, medium, and fine clay subfractions isolated from samples of surface soil (0–15 cm) collected from two different agricultural fields and a native prairie in north central Iowa, USA

Soil	Size fraction (μm)	Element content (g kg^{-1} soil clay)		C:N	C minimum mean residence time (^{14}C YBP or % MC) ^a	C mineralized during incubation (% C, w/w)
		N	C			
Native prairie	Whole clay	5.0	75.8	15		2.09 ± 0.07
	Coarse	3.1	64.6	21	380 ± 40 YBP	1.09 ± 0.11
	Medium	6.8	79.5	12		2.01 ± 0.04
	Fine	5.1	71.1	14	112.9 ± 0.6 %MC	2.09 ± 0.21
Field 1	Whole clay	4.6	67.7	15		2.21 ± 0.09
	Coarse	2.7	63.8	24	670 ± 40 YBP	0.69 ± 0.03
	Medium	5.5	73.8	13		1.61 ± 0.08
	Fine	4.3	56.4	13	101.0 ± 0.5 %MC	1.55 ± 0.10
Field 2	Whole clay	3.4	62.7	18		1.89 ± 0.04
	Coarse	2.8	67.2	24	296 ± 40 YBP or 60 ± 40 YBP ^b	0.35 ± 0.08
	Medium	4.9	77.2	16		0.89 ± 0.04
	Fine	2.4	45.4	19	106.6 ± 0.5 %MC	1.61 ± 0.05

^a $\delta^{13}\text{C}$ corrected radiocarbon age in years before present (YBP) or % modern C (MC) relative to a 1950 oxalic acid standard.

^b The level of ^{14}C in this sample is consistent with two different ^{14}C age ranges.

focus hereafter is on the organic material that physically separates with the whole clay (47 to 60% of total SOC) and is commonly assumed to be composed of humic substances.

The various soil clay subfractions isolated from these soils all contain similar levels of organic C (Table 2), however the C:N ratios of the coarse clay subfractions (21 to 24) are substantially higher than those of the medium or fine clay subfractions (12 to 16 and 13 to 19, respectively). The clay mineralogy of the subfractions is also distinctive (Fig. 1). The fine clay is dominated by smectite with lesser amounts of a low-charge, randomly interstratified illite and smectite (I/S) (Laird et al., 1991). The medium clay is dominated by randomly interstratified I/S with lesser amounts of kaolinite and discrete illite (1.0-nm illite). And, the coarse clay is dominated by quartz with lesser amounts of kaolinite, 1.0-nm illite, and feldspar. The physical removal of virtually all of the smectite and randomly

interstratified I/S from the coarse clay subfraction is a unique and critical feature of our fractionation procedure.

The results of the incubation study (Table 2) indicate that the organic C in the fine and medium clay subfractions was significantly ($P > 0.01$) more bioavailable than was organic C in the coarse clay subfractions. The procedure used to physically separate the coarse, medium, and fine clay subfractions is aggressive, and any organic material that was solubilized during the procedure would have been lost. An estimate of the amount

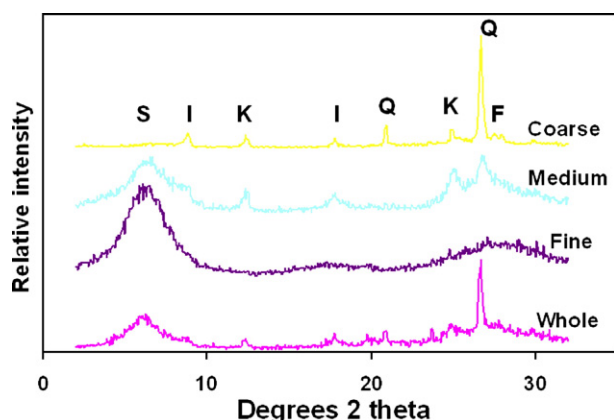


Fig. 1. X-ray diffraction patterns of the whole clay fraction and the coarse, medium and fine clay subfractions isolated from the surface soil (0–15 cm) of an agricultural soil (field 1). All samples were Ca-saturated, washed free of excess salt, prepared as oriented specimens on glass slides, air-dried and analyzed using Cu-K α radiation. Major peak positions for smectite and randomly interstratified I/S (S), 10 Å-illite (I), kaolinite (K), quartz (Q), and feldspars (F) are indicated.

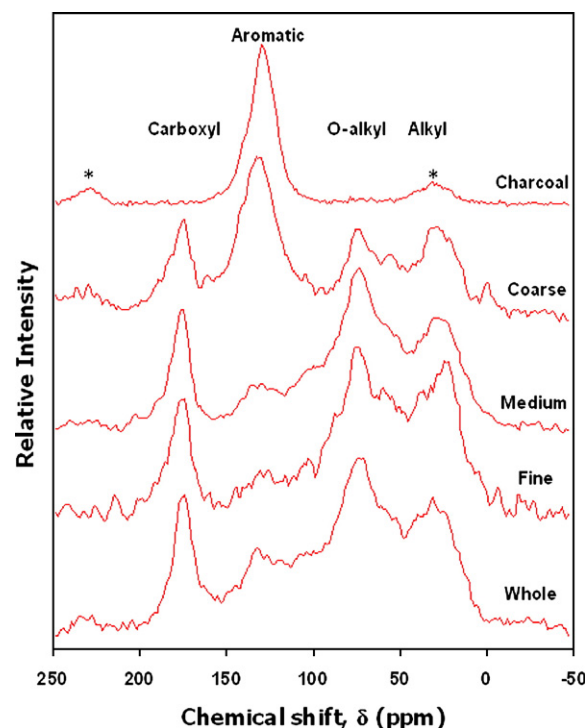


Fig. 2. Solid-state ^{13}C CP-MAS NMR spectra of the whole clay and coarse, medium, and fine clay subfractions from an agricultural soil (field 1) and a charcoal reference sample. Stars indicate spinning side bands on the charcoal spectra only.

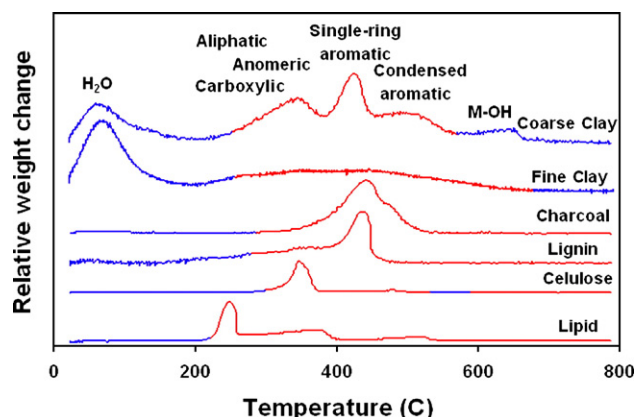


Fig. 3. Differential thermal gravimetric analysis of the coarse and fine clay subfractions of an agricultural soil (field 1) and reference samples of charcoal, lignin, and cellulose. Exothermic regions are in red and endothermic regions are in blue. Samples were run in air at 10 °C per min. Clay subfractions were run as is, reference organic materials were physically mixed (1:10 w:w) with silt-size quartz. M-OH indicates dehydroxylation of clay minerals.

of organic C lost during the fractionation is obtained by comparing the measured organic C values for the whole clay with the sum of organic C for the subfractions weighted by their respective masses (Table 1). This comparison indicates that 2.6 to 5.6% of the C was lost during the fractionation procedure. By comparison, the weighted amount of C mineralized during incubations of the coarse, medium, and fine clay subfractions was 22 to 49% less than the measured amount of C mineralized for the whole clay samples (Table 2). These results suggest that the dissolved organic matter lost during the physical fractionation made a substantial contribution to the bioavailable C in the whole clay samples. Thus the incubation results for the coarse, medium, and fine clay subfractions should be viewed only as a relative measure of bioavailability of organic C that physically separated with these subfractions.

Our radiocarbon dating results indicate minimum mean residence times for C in the coarse clay subfractions ranging from 60 to 670 YBP. By contrast, all of the fine clay subfractions contained greater than 100% modern C relative to the level of ^{14}C in a 1950 oxalic acid standard (Table 2). The radiocarbon dates reported in Table 2 should be regarded as “minimum mean residence times” for the organic C for two reasons. First, SOM is

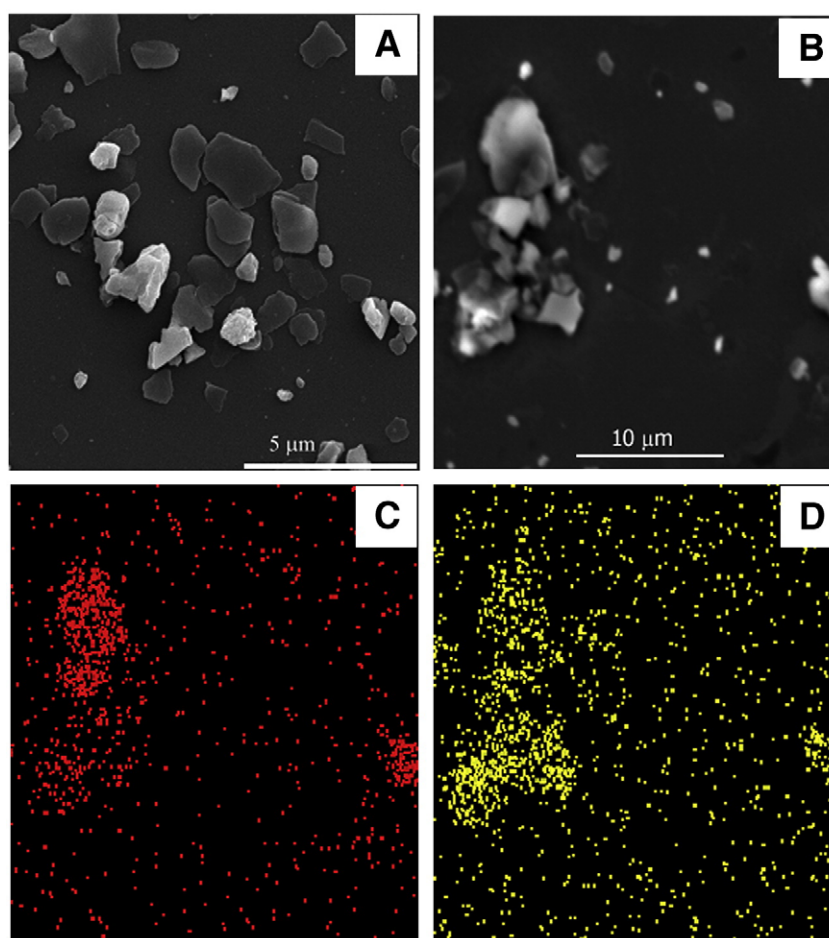


Fig. 4. SEM images and EDX dot maps of the coarse clay subfraction separated from an agricultural soil (field 1). (A) High resolution image showing the morphology of discrete particles taken with the final aperture in and using a sputter coated sample. (B) low resolution image of the $<1.5 \text{ g cm}^{-3}$ density fraction take with the final aperture out on a non-sputter coating sample. (C) Energy dispersive X-ray dot map of the same region shown in B showing the distribution of carbon. And (D) Energy dispersive X-ray dot map of the same region shown in B showing the distribution of oxygen.

dynamic as new C is constantly added and older C is constantly being lost from the soil through mineralization. Second, these soils were undoubtedly contaminated with ^{14}C during the era of atmospheric nuclear testing (1945–1980).

Previously, we demonstrated that new C derived from plant tissue preferentially accumulates in the fine clay subfraction relative to the coarse clay subfraction during 360-d incubations of ^{14}C -labeled residue in soil (Gonzalez and Laird, 2003). The previous study was conducted using the same fractionation scheme that we have described here but a different soil. Taken together, the incubation study, the radiocarbon dating, and the ^{14}C -labeled residue study strongly suggest that the organic material that separates with the fine and medium clay subfractions is biologically more active than the organic material that separates with the coarse clay subfraction.

Solid-state ^{13}C -NMR analysis of clay-humic complexes is challenging, as both total organic C and natural abundance ^{13}C levels are low and untreated samples may contain significant levels of ferromagnetic elements (primarily Fe) that degrade the NMR signal. Although the signal-to-noise ratio is low, our ^{13}C -CPMAS-NMR spectra were obtained directly on the physically isolated soil clay subfractions. The spectra for the coarse clay subfractions are dominated by a prominent aromatic C peak centered at 127 ppm, with smaller peaks for carboxyl C at

171 ppm, O-alkyl C at 77 ppm, and alkyl C at 29 ppm (Fig. 2). By contrast, the ^{13}C -CPMAS-NMR spectra for the medium and fine clay subfractions have large peaks for alkyl, O-alkyl, and carboxyl C with little evidence of aromatic C. The spectra for the whole clay may be viewed as a weighted average of the three subfractions with contributions from alkyl, O-alkyl, carboxyl, and aromatic C. The spectrum for the charcoal sample is included as a reference. Spinning side bands (SSB) off the aromatic peak are evident at 227 and 30 ppm on the charcoal spectrum. Under conditions of the analysis, SSB from the aromatic C peak may interfere with the alkyl C peak but are otherwise out of the region of interest. Previous studies have found relatively lower aromatic C content in whole clay fractions than in sand and silt fractions (Christensen, 2001). Our results demonstrate that most of the aromatic C in the whole clay can be physically separated from the aliphatic-rich humic material by exhaustive dispersion-centrifugation-decantation.

The NMR interpretations were supported by differential thermal gravimetric (DTG) analysis. The thermograph for the coarse clay subfraction (Fig. 3) shows three distinct exotherms centered at 350, 430, and 505 °C, indicating 41, 32, and 27% relative mass loss, which we attribute to aliphatic-anomeric-carboxylic, single-ring aromatic, and condensed aromatic moieties, respectively. By contrast, the thermograph for the

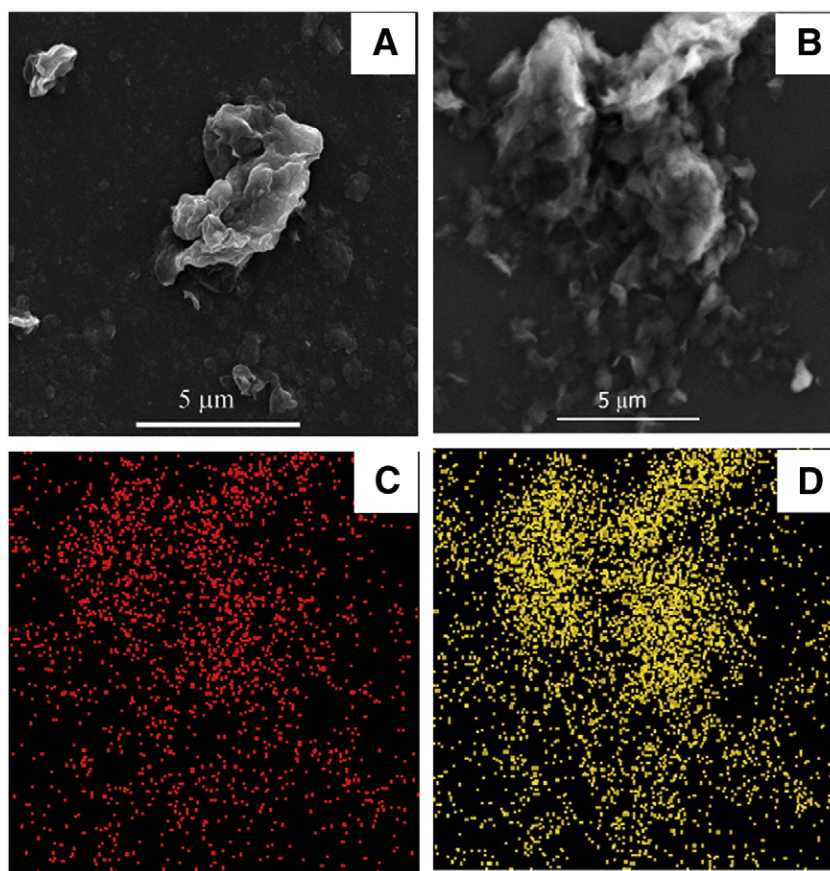


Fig. 5. SEM images and EDX dot maps of the fine clay subfraction separated from an agricultural soil (field 1). (A) High resolution image showing the morphology of smectite quasicrystal taken with the final aperture in and using a sputter coated sample. (B) Low resolution image of a smectite quasicrystal take with the final aperture out on a non-sputter coating sample. (C) Energy dispersive X-ray dot map of the same region shown in B showing the distribution of carbon. And (D) Energy dispersive X-ray dot map of the same region shown in B showing the distribution of oxygen.

fine clay subfraction has a broad, nearly featureless, exotherm beginning at 230 °C and extending to 600 °C, indicative of a heterogeneous mixture of aliphatic, anomeric, and carboxylic organic materials. Thermal analysis for a series of reference materials showed major peaks consistent with our interpretations (Fig. 3). During thermal analysis pyrolysis products formed by incomplete combustion of a component may produce “ghost peaks” at higher temperatures. The small peak at 390 °C in the thermogram for the reference lipid sample is an example of such an artifact. Problems with ghost peaks may partially explain the very broad nature of the exotherm for the fine clay subfraction.

Scanning electron microscopy of the Ca-saturated coarse clay subfractions revealed discrete particles that were approximately 0.2 to 5 µm in diameter (Fig. 4A). From the XRD analysis (Fig. 1), we know that most of these particles are quartz, 1.0-nm illite, kaolinite, and feldspar. However, the sample also contains 63.8 g kg⁻¹ C (Table 2), and thus we infer that some of the discrete particles must be C rich. The <1.5 g cm⁻³ density fraction of the coarse clay subfraction from field 1 was highly enriched with C (192.2±0.8 g kg⁻¹ organic C). SEM coupled with EDX analysis of the <1.5 g cm⁻³ density fraction revealed only discrete particles (Fig. 4B). Many of these discrete particles were highly enriched with C and have relatively lower O levels (compare the relative intensity of the C and O signals in Fig. 4C and D). The combined XRD, NMR, DTG, SEM, and EDX evidence lead us to infer that the C-enriched particles in the <1.5 g cm⁻³ density fraction of the coarse clay subfraction are charcoal. Organic C in the fine and medium clay subfractions, by contrast, was associated with diffuse filamentous material (Laird, 2001) that was both bound to Ca-saturated smectite quasicrystals and scattered on the surface of the specimen in small amorphous clumps (Fig. 5A–D). We infer that this diffuse material is the true humic component of our soils.

In summary, our data indicate that two types of organic material can be physically separated from these soil clays. One phase consists of older, biologically more stable, discrete, roughly equi-dimensional particles (0.2–5.0 µm) that are dominated by both single- and condensed-ring aromatic C (~18% of C in the whole clay and ~9% of total SOC) with lesser amounts of aliphatic-anomeric-carboxylic C (~12% of C in the whole clay and ~7% of total SOC). Properties of this phase are consistent with those of aged charcoal that has accumulated biogenic organic C either by adsorbing dissolved organic C from the soil solution or by direct deposition from microbes living within the charcoal particles. A significant charcoal component in these Iowa soils is highly plausible given the stability of charcoal in soil environments and a legacy of periodic prairie fires during 10,000 years of post-glacial soil development under prairie vegetation. Because soil charcoal is pyrogenic we believe it should not be included as a component of HS. The second type, approximately 70% of the C in the whole clay (38% of total SOC), consists of diffuse, filamentous organic materials that physically separate with the smectite and randomly interstratified I/S in the fine and medium clay subfractions. This organic material is primarily a mixture of aliphatic, anomeric, and carboxylic C and is both relatively young and biologically available. We conclude that this material

is the biogenic humic component of our soils. The paucity of aromatic C in this biogenic humic material is consistent with previous studies that found little aromatic C in HS newly formed from ¹³C-labeled glucose (Baldock et al., 1990) and inconsistent with the traditional heteropolymer model of HS that assumes an aromatic backbone.

Biogenic aromatic C is found in plant and fungal tissue and can also be identified in the particulate organic matter that separates with the sand and silt size fractions. That little biogenic aromatic C is present in the humified soil organic matter that separates with our medium and fine clay subfractions, suggests that biogenic aromatic C was either selectively decomposed during humification or selectively adsorbed by the soil charcoal and hence not distinguished from the pyrogenic aromatic C.

Acknowledgements

The authors thank Pierce Fleming for analytical help and Tracy Pepper for help with electron microscopy. Partial support for this work is from the Department of Agronomy at Iowa State University, Iowa State Agriculture and Home Economics Experiment Station.

References

- Almendros, G., Knicker, H., Gonzalez-Vila, F.J., 2003. Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state ¹³C- and ¹⁵N-NMR spectroscopy. *Organic Geochemistry* 34, 1559–1568.
- Baldock, J.A., Oades, J.M., Vassallo, A.M., Wilson, M.A., 1990. Solid state CP/MAS ¹³C N.M.R. analysis of particle size and density fractions of soil incubated with uniformly labelled ¹³C-glucose. *Australian Journal of Soil Research* 28, 193–212.
- Brodowski, S., Amelung, W., Haumaier, L., Abetz, C., Zech, W., 2005. Morphological and chemical properties of black carbon in physical soil fractions as revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy. *Geoderma* 128, 116–129.
- Burdon, J., 2001. Are the traditional concepts of the structures of humic substances realistic? *Soil Science* 166, 752–769.
- Cambardella, C.A., Elliott, E.T., 1992. Particulate soil organic matter across a grassland cultivation sequence. *Soil Science Society of America Journal* 56, 777–783.
- Christensen, B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. *European Journal of Soil Science* 52, 345–353.
- Drinkwater, L.E., Cambardella, C.A., Reed, J.D., Rice, C.W., 1996. Potentially mineralizable nitrogen as a property of biologically active soil nitrogen. In: Doran, J.W., Jones, A.J. (Eds.), *Methods for Assessing Soil Quality*. SSSA Spec. Publ. 49. SSSA, Madison, WI, pp. 217–229.
- Fernandes, M.B., Skjemstad, J.O., Johnson, B.B., Wells, J.D., Brooks, P., 2003. Characterization of carbonaceous combustion residues I. Morphological, elemental and spectroscopic features. *Chemosphere* 51, 785–795.
- Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A. (Ed.), *Methods of Soil Analysis, Part 1, Physical and Mineralogical Methods*, Second Edition. Soil Science Society of America, Madison, WI, pp. 383–412.
- Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal — a review. *Biology and Fertility of Soils* 35, 219–230.
- Gonzalez, J.M., Laird, D.A., 2003. Carbon sequestration in clay mineral fractions from ¹⁴C-labeled plant residues. *Soil Science Society of America Journal* 67, 1715–1720.
- Kramer, R.W., Kujawinski, E.B., Hatcher, P.G., 2004. Identification of black carbon derived structures in a volcanic ash soil humic acid by Fourier

- transform ion cyclotron resonance mass spectrometry. *Environmental Science and Technology* 38, 3387–3395.
- Laird, D.A., 2001. Nature of clay-humic complexes in an agricultural soil: (II) scanning electron microscopy analyses. *Soil Science Society of America Journal* 65, 1419–1425.
- Laird, D.A., Barak, P., Nater, E.A., Dowdy, R.H., 1991. Chemistry of smectitic and illitic phases in interstratified soil smectite. *Soil Science Society of America Journal* 55, 1499–1504.
- Laird, D.A., Martens, D.A., Kingery, W.L., 2001. Nature of clay-humic complexes in an agricultural soil: (I) chemical, biochemical, and spectroscopic analyses. *Soil Science Society of America Journal* 65, 1413–1418.
- Lal, R., 2004. Soil carbon sequestration to mitigate climate change. *Geoderma* 123, 1–22.
- Mao, J.D., Fang, X., Schmidt-Rohr, K., Carmo, A.M., Hundal, L.S., and Thompson, M.L., in press. Molecular-scale heterogeneity of humic acid in particle-size fractions of two Iowa soils. *Geoderma*.
- Orlov, D.S., 1985. *Humus acids of soils*. Oxonian Press, New Delhi. English translation.
- Piccolo, A., 2001. The supramolecular structure of humic substances. *Soil Science* 166, 810–832.
- Schulten, H.R., Schnitzer, M., 1993. A state of the art structural concept for humic substances. *Naturwissenschaften* 80, 29–30.
- Simpson, A.J., 2002. Determining the molecular weight, aggregation, structures and interactions of natural organic matter using diffusion ordered spectroscopy. *Magnetic Resonance in Chemistry* 40, S72–S82.
- Simpson, M.J., Hatcher, P.G., 2004. Determination of black carbon in natural organic matter by chemical oxidation and solid-state ^{13}C nuclear magnetic resonance spectroscopy. *Organic Geochemistry* 35, 923–935.
- Skjemstad, J.O., Reicosky, D.C., Wilts, A.R., McGowan, J.A., 2002. Charcoal Carbon in U.S. Agricultural Soils. *Soil Science Society of America Journal* 66, 1249–1255.
- Steelink, C., 1985. Implications of elemental characteristics of humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (Eds.), *Humic Substances in Soil, Sediment, and Water*. Wiley, New York, pp. 457–476.
- Stevenson, F.J., 1994. *Humus Chemistry: Genesis, Composition, Reactions*, ed. 2. John Wiley & Sons, Inc., New York.
- Swift, R.S., 1996. Organic matter characterization. In: Sparks, D.L., et al. (Ed.), *Methods of Soil Analysis: Part 3 — Chemical Methods*. Soil Science Society of America, Madison, WI, pp. 1011–1070.
- Wershaw, R.L., Leenheer, J.A., Cox, L.G., 2005. U.S. Geological Survey Scientific Investigations Report. 2005–5108.
- Zhang, H., Thompson, M.L., Sandor, J.A., 1988. Compositional differences in organic matter among cultivated and uncultivated Argiudolls and Hapludalfs derived from loess. *Soil Science Society of America Journal* 52, 216–222.